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Postopek za pridobivanje inhibitorjev HMG-CoA visoke čistosti

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LEK, Pharmaceutical and
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Title of the invention

Process for the obtaining of HMG-CoA reductase inhibitors of high purity

C 07D 309/30

Background of the invention

Lovastatin, pravastatin, mevastatin, their derivatives and analogs are known as HMG-CoA reductase inhibitors and are used as antihypercholesterolemic agents. They are produced by fermentation using microorganisms of different species identified as species belonging to *Aspergillus*, *Monascus*, *Nocardia*, *Amycolatopsis, Mucor* or *Penicillium* genus.

Purity of the active ingredient is important factor for the manufacturing of a safe and effective pharmaceutical. The highest possible purity of the product is especially important if the pharmaceutical product should be taken for a longer period as it is the case in the treatment or the preventing of a high plasma cholesterol. The accumulation of the impurities from the pharmaceuticals of lower purity can cause many side effects during the medical treatment.

The processes for the isolation and purification of the antihypercholesterolemic agents disclosed in the earlier patent applications comprise different combinations of extraction, chromatography, lactonization and crystallization methods. The purity of the final product obtained by these procedures is lower than 99.6%. Obtaining the product of higher purity by use of these methods is possible, but the yield of the desired product is then unacceptably low for their use that methods in a large industrial scale.

The isolation process disclosed in patent application WO 92/16276 provides the solution for obtaining HMG-CoA reductase inhibitors of purity

higher than 99.5%, but the use of highly sophisticated industrial high performance liquid chromatography (HPLC) equipment is required. According to the WO 92/16276 the crude HMG-CoA reductase inhibitor of approximately 85% or higher purity is dissolved in an organic solvent or in a solution of organic solvent and water. The mixture is then buffered to a pH between 2 and 9 and placed on an HPLC column. After the HMG-CoA reductase inhibitor peak of interest is collected, a portion of solvent is removed and then water is added or alternatively two-thirds of the solvent mixture are removed to crystallize the HMG-CoA reductase inhibitor. At the end the purity of the product achieved by this process is really at least 99.5% with yield of approximately 90%.

Detailed description of the invention

The present invention relates to a new industrial process for isolation and purification of HMG-CoA reductase inhibitors of purity higher than 99.6% from a fermentation broth. To achieve this goal an extensive study of the chemical compounds produced during the fermentation using the different species of microorganisms belonging to *Aspergillus*, *Monascus*, *Nocardia*, *Mucor*, *Amycolatopsis* or *Penicillium* genus, their chemical properties and their behavior in the different solvents at different pH was done. The process of the present invention comprises:

a) dissolvation of the HMG-CoA reductase inhibitor from the mycelium biomass at pH value between 9.5 and 13 into fermentation liquor
b) treatment of the whole broth with mineral acid to pH value between 7.5 and 8.5
c) removal of the mycelium from the broth by means of filtration to obtain clarified broth filtrate and concentration of said filtrate to a lower volume

d) acidifying of the concentrate to pH value between 5.5 and 7.5 with mineral acid followed by extraction of the HMG-CoA reductase inhibitor with ethyl acetate

e) optionally lactonization

f) optionally adsorption chromatography with mixture of acetonitrile and water as mobile phase

g) crystallization of the HMG-CoA reductase inhibitor from water miscible organic solvent

h) crystallization of the HMG-CoA reductase inhibitor from water immiscible organic solvent

The order of the last two steps of the isolation process is not important. The adsorption chromatography may be also replaced with crystallization of the HMG-CoA reductase inhibitor from ethyl acetate or with some extraction tehnic.

HMG-CoA reductase inhibitors are intra- and extracellular products and therefor they should be dissolved from the mycelium into fermentation liquor. The method for dissolvation disclosed in patent application WO 97/20834 comprises treatment of fermentation broth with alkaline base to pH 11.5 and stirring for three hours. The WO 97/06128 teaches that dissolvation may be done with alkalifying of the fermentation broth to pH between 10 and 13. Also temperature between 60 and 95 C is performed. HMG-CoA reductase inhibitors may be very efficiently dissolved from the mycelium at pH higher than 9, but too long exposure to so rigorous condition causes the degradation of ester bond between hydroxyl group on naphthalene skeleton and carboxylic acid. Equilibrium between HMG-CoA reductase inhibitors and deacylated HMG-CoA reductase inhibitors shifts at more rigorous conditions to deacylated products. We have unexpectedly found out is that the efficiency of dissolvation carried out at room temperature for about 10 minutes at pH between 9.5 and 13, most preferably between 9.5 and 11.5, is equal to efficiency achieved by less economic and more time consuming methods carried out at higher temperatures

described in earlier patent applications. The dissolvation may be carried out also at pH lower than 6, but the use of huge amount of organic solvents is necessary.

After the dissolvation of the HMG-CoA reductase inhibitor the fermentation broth is treated with mineral acid to pH value between 7.5 and 8.5. Suitable mineral acids are phosphoric, sulfuric and hydrochloric acid. HMG-CoA reductase inhibitors are stabile in this range of pH and the fermentation broth can be also stored after this step for a while if that is necessary.

The mycelium is removed from the fermentation broth by means of filtration. As a filtration tehnic beside classic filtration may be used also micro-, ultra- and diafiltration. The filtrate is than concentrated to lower volume, most preferably five to ten times by means of reverse osmosis or some other methods for lowering volume.

The said concentrate is acidified by mineral acid to pH value between 5.5 and 7.5 with mineral acid and HMG-CoA reductase inhibitor is then extracted from the said concentrate with ethyl acetate. The ratio between distribution coefficients of HMG-CoA reductase inhibitors and ethyl acetate soluble impurities is the highest at pH value between 5.5 and pH 7.5 and a part of polar impurities is already removed at this step. The extraction carried out at pH value lower than 4 is more efficient, because of higher distribution coefficient of HMG-CoA reductase inhibitors, but it results in high level of polar impurities. The distribution coefficients of ethyl acetate soluble impurities are at that pH value also high. (Fig.1). The extraction into ethyl acetate carried out at pH value between 5.5 and 7.5 results in lower level of polar impurities because of their low distribution coefficients. Worse distribution of HMG-CoA reductase inhibitors from the concentrate into ethyl acetate at that pH value can be easily compensate with longer counter current extraction column.

The resulted ethyl acetate extract is then concentrated and HMG-CoA reductase inhibitor is lactonized by contacting with catalytic amount of mineral or organic acid, most preferably triflouracetic acid (TFA). At pH between 5.5 and 7.5 is the major part of the HMG-CoA reductase inhibitor in free acid

form therefor the concentration and lactonization may be omitted if HMG-CoA reductase inhibitor is not used in the pharmaceutical as a lactone. After lactonization the ethyl acetate is removed by vaporization and the raw HMG-CoA reductase inhibitor is obtained.

Optionally is the raw HMG-CoA reductase inhibitor dissolved in pure acetonitrile or mixture acetonitrile/water with at least 30% volume/volume (v/v) of acetonitrile and the resulting solution is placed on an adsorption chromatography column. The column packing include, but are not limited to octylsilane, dimethylsilane, octadecylsilane, cyano-silane, polystyrenedivinylbenzene copolymer or acryl stationary phase. The adsorbed compounds are eluted with acetonitrile/water gradient. The HMG-CoA reductase inhibitor peak of interest is collected and the acetonitrile is removed to crystallize the HMG-CoA reductase inhibitor. The purity of crystallized crude HMG-CoA reductase inhibitors is between 80% and 92% depends on impurity profile in the fermentation broth. The adsorption chromatography may be also replaced by methods of extraction or crystallization.

We have unexpectedly found out that the crystallization of HMG-CoA reductase inhibitors from water miscible organic solvent like acetone followed by further recrystallizations with the same solvent can remove only a minor part of nonpolar and a major part of polar impurities and the crystallization from water immiscible organic solvent like ethyl acetate followed by further recrystallizations from the same solvent remove in major nonpolar impurities. The last fact are clearly evident from HPLC diagrams of crude HMG-CoA reductase inhibitor (Fig.2), HMG-CoA reductase inhibitor after the crystallization from acetone (Fig.3) and HMG-CoA reductase inhibitor obtained by the crystallization from acetone and further recrystallized from ethyl acetate (Fig.4). According to this unexpected recognition the last step of present invention comprising combined crystallization from water immiscible and water miscible organic solvent and can not be omitted in our process for achieving HMG-CoA reductase inhibitors of high purity. Order of both crystallizations may be also inverse.

According to the process of the present invention the crystals of crude HMG-CoA reductase inhibitor are dissolved in 100% water miscible organic solvent, most preferably acetone or lower alcohol and then water is added to let HMG-CoA reductase inhibitor to crystallize. This procedure may be repeated again, if necessary, for example from one to four times depends on the purity of the starting crude material.

Crystals obtained by crystallization from water miscible organic solvent are dissolved in pure water immiscible organic solvent, like ethyl acetate to the concentration of between 10 and 35 g/l, most preferably between 15 and 25 g/l. After the removal of one-third to three-fourth of solvent the HMG-CoA reductase inhibitor crystallizes. Crystallization from water immiscible organic solvent may be repeated again, if necessary, for example for one to three times depends on the purity of the product obtained by crystallization from water miscible organic solvent. The crystallized HMG-CoA reductase inhibitor is than filtered and dried to yield a product of purity of at least 99.6%.

The present invention is directed also to a process for the isolation of HMG-CoA reductase inhibitors comprising

a) dissolvation of the HMG-CoA reductase inhibitor from the biomass at pH value higher than 9.5
b) treatment of the whole broth with mineral acid to pH value between 7.5 and 8.5
c) removal of the mycelium from the fermentation broth by means of filtration to obtain clarified broth filtrate and concentration to lower volume
d) acidifying of the concentrate with mineral acid to pH value between 5.5 and 7.5 and extraction of the HMG-CoA reductase inhibitor the with ethyl acetate

and may be used as a first step of any process for isolation and purification of HMG-CoA reductase inhibitors described in prior patent applications.

The last step of combined crystallization from water miscible and water immiscible organic solvent may be employed also as a final polishing step of

any other process for isolation and purification of HMG-CoA reductase inhibitors.

The following examples illustrate the process of the instant invention and are not be considered as limiting the invention set forth in claims appended hereto.

EXAMPLES

Example 1

Fermentation broth (160 l) with concentration of lovastatin 1g/l obtained by fermentation with *Aspergillus terreus* ATCC 20542 was placed into the vessel (400 l) and adjusted to pH 10 with 1M aqueous sodium hydroxide solution. After 10 minutes of intensive stirring the broth was adjusted to pH 9 with 1M sulfuric acid solution and the biomass was filtered off. The filtrate was then acidified with 1M sulfuric acid solution to pH 6.5. 160 l of ethyl acetate was added to filtrate and the obtained mixture was stirred for 20 min. The aqueous and ethyl acetate phases were separated by extraction centrifuge. The ethyl acetate extract was concentrated in rotary evaporator to volume of 14 l. The concentration of the lovastatin in the free acid form in the ethyl acetate concentrate amounted to 10 g/l.

The ethyl acetate concentrate (14 l) was then placed into reactor (40 l) and lactonized. The lactonization was initiated by catalytic amount of TFA (0.5 ml of TFA/ 1 l of concentrate). The lactonization procedure last at 40 C for two hours. The concentrate was washed after the lactonizaton two times with 14 l of 5% ammonium hydrogen carbonate aqueous elution. The aqueous phase was discharged, the organic phase was further concentrated to dry in rotary evaporator. The resulted oily product (1.5 l) contained 133 g of lovastatin.

The obtained oily product (161 ml) was dissolved in 80 ml of acetonitrile and loaded on a chromatography column (80 cm, 3.6 cm) filled with XAD-16 (XAD-16 is commercial name of company Rohm&Hass, 20-50 mesh). The

column was eluted first with 40:60 acetonitrile/water (pH 3, adjusted by hydrochloric acid) at rate of 75 ml/min. Elution was monitored by UV detector (236 nm) and after first drop of absorption the elution of the column with 55:45 acetonitrile/water (pH 3, adjusted by hydrochloric acid) was started. The main fraction was collected and after the fall of the absorption the column was washed with 80:20 acetonitrile/water (pH 3, adjusted by hydrochloric). The acetonitrile was removed from the main fraction by rotary evaporator (50 C, 150 mbar) and the resulted crystals were filtered off. Mass of crystals was 24.5g and the content of lovastatin was 50% weight/weight (w/w). HPLC purity was 92.5%.

Resulted crystals (24g) were dissolved in 350 ml acetone and 700 ml of water was added under the continuos stirring. The mixture was placed on 4 C for 30 minutes. Obtained crystals were filtered off and dried in vacuo at room temperature. Mass of crystals was 12.7 g with the content 90% w/w of lovastatin. HPLC purity was 98.8%

The crystallization from acetone was repeated again under the same condition and 11.3 g of crystals with 97% w/w of lovastatin were obtained. HPLC purity was 99.4%.

The crystals (11.3 g) obtained after the second crystallization from acetone were dissolved in 700 ml of ethyl acetate and the ethyl acetate was evaporated in vacuo to the concentration of lovastatin 70 g/l. The concentrate was placed on 8 C for one hour. Resulted crystals of lovastatin were filtered off and then dried in vacuo. Mass of crystals was 9.4g with 99.6% w/w content of lovastatin. HPLC purity was 99.7%

Example 2

Lovastatin crystals (3g), isolated after the XAD-adsorption chromatography as described in Example 1, were dissolved in 170 ml of ethyl acetate. The ethyl acetate was evaporated in vacuo (200 mbar) at 50 C to 35 ml. The concentrate

was placed on 10 C for one hour. Resulted crystals of lovastatin were filtered off and then dried in vacuo. Mass of crystals was 2.1g with 96% w/w content of lovastatin. HPLC purity was 99.0%.

Obtained crystals (2.1g) were dissolved in 50 ml acetone and 85 ml of water was added. The mixture was placed than on 10 C for 30 minutes and the crystals were filtered off and dried in vacuo at 40 C. Mass of resulted crystals was 1.9 g with the 99% w/w of lovastatin. HPLC purity was 99.8%

What is claimed is:

1. A process for the isolation and purification of HMG-CoA reductase inhibitors of purity higher than 99.6% which comprises:

a) dissolvation of the HMG-CoA reductase inhibitor from the mycelium biomass at pH value between 9.5 and 13 into fermentation liquor

b) treatment of the whole broth with mineral acid to pH value between 7.5 and 8.5

c) removal of the mycelium from the broth by means of filtration to obtain clarified broth filtrate and concentration of said filtrate to a lower volume

d) acidifying of the concentrate to pH value between 5.5 and 7.5 with mineral acid followed by extraction of the HMG-CoA reductase inhibitor with ethyl acetate

e) optionally lactonization

f) adsorption chromatography with mixture of acetonitrile and water as mobile phase

g) crystallization of the HMG-CoA reductase inhibitor from water miscible organic solvent

h) crystallization of the HMG-CoA reductase inhibitor from water immiscible organic solvent

2. A process for the isolation and purification of HMG-CoA reductase inhibitors of purity higher than 99.6% which comprises:

a) dissolvation of the HMG-CoA reductase inhibitor from the mycelium biomass at pH value between 9.5 and 13 into fermentation liquor

b) treatment of the whole broth with mineral acid to pH value between 7.5 and 8.5

c) removal of the mycelium from the broth by means of filtration to obtain clarified broth filtrate and concentration of said filtrate to a lower volume

d) acidifying of the concentrate to pH value between 5.5 and 7.5 with mineral acid followed by extraction of the HMG-CoA reductase inhibitor with ethyl acetate

e) optionally lactonization

f) crystallization of the HMG-CoA reductase inhibitor from ethyl acetate

g) crystallization of the HMG-CoA reductase inhibitor from water miscible organic solvent

h) crystallization of the HMG-CoA reductase inhibitor from water immiscible organic solvent

3. A process for the isolation and purification of HMG-CoA reductase inhibitors of purity higher than 99.6% which comprises:

a) dissolvation of the HMG-CoA reductase inhibitor from the mycelium biomass at pH value between 9.5 and 13 into fermentation liquor

b) treatment of the whole broth with mineral acid to pH value between 7.5 and 8.5

c) removal of the mycelium from the broth by means of filtration to obtain clarified broth filtrate and concentration of said filtrate to a lower volume

d) acidifying of the concentrate to pH value between 5.5 and 7.5 with mineral acid followed by extraction of the HMG-CoA reductase inhibitor with ethyl acetate

4. A process for the isolation and purification of HMG-CoA reductase inhibitors of purity higher than 99.6% which comprises the combined crystallization from water miscible organic solvent and crystallization from water immiscible organic solvent as a final polishing step.

Fig.1

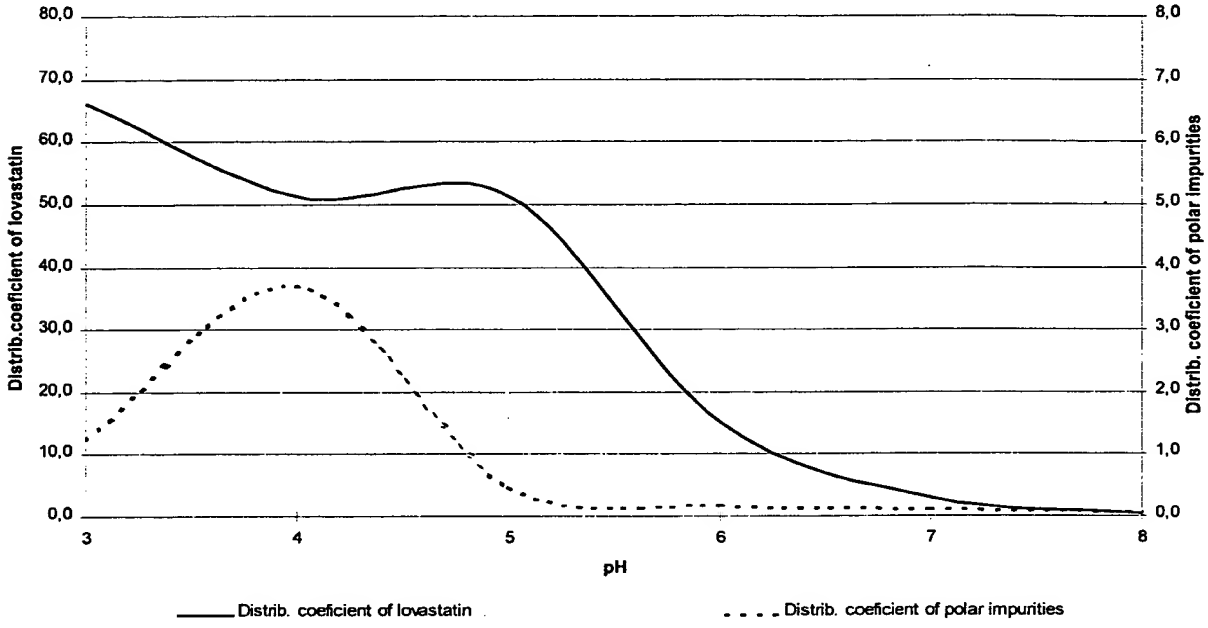
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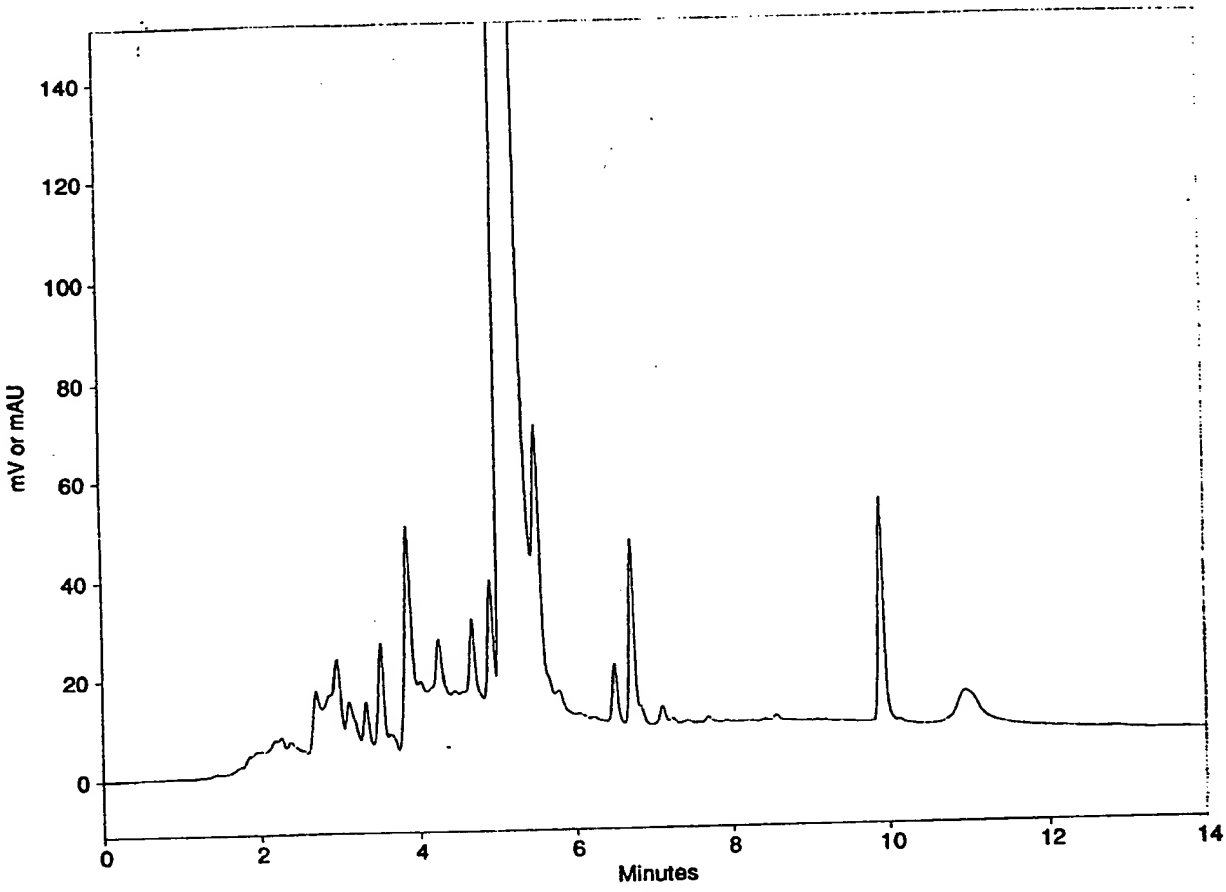
Fig.2



LEK d.d.

Alenka Košak, dipl.ing.

Head of Industrial Property Department

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Fig.3

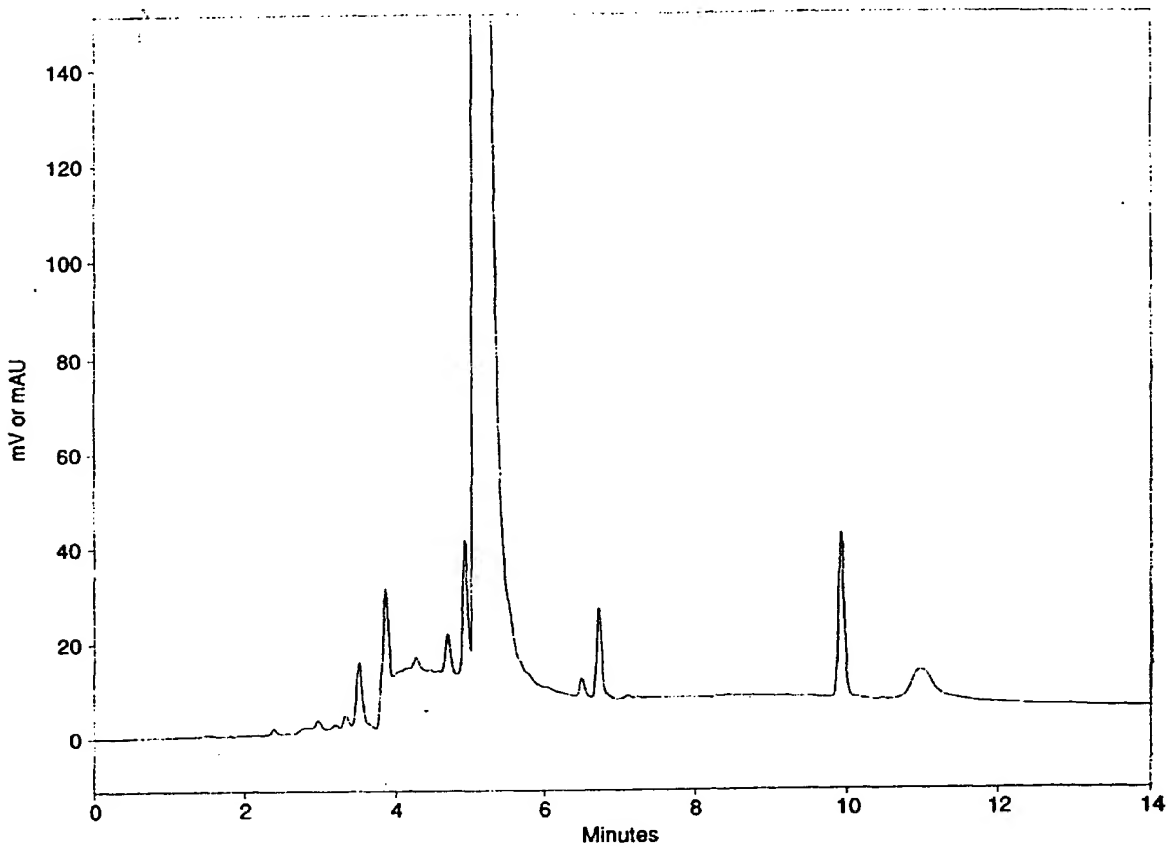
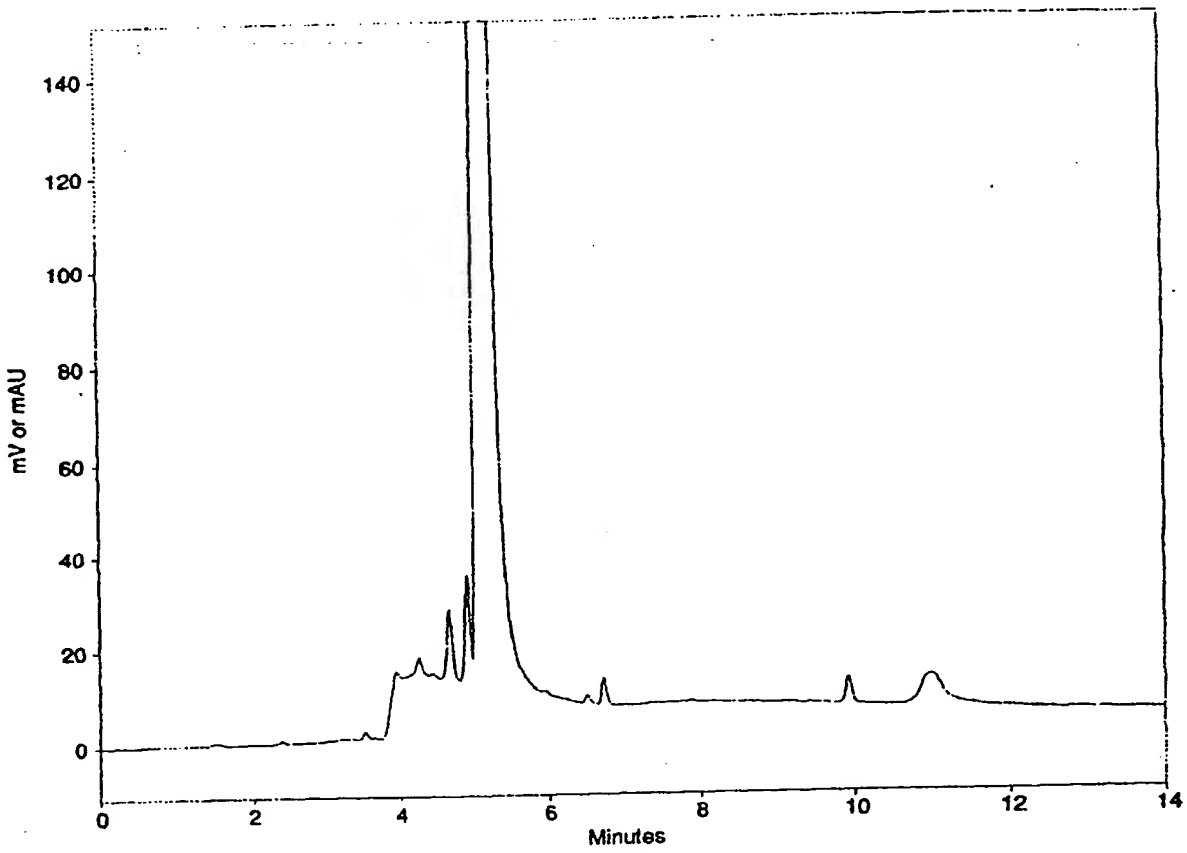


Fig.4



Abstract

HMG-CoA reductase inhibitors are used in pharmacy as antihypercholesterolemic agents. They are produced by fermentation using microorganisms of different species identified as species belonging to *Aspergillus*, *Monascüs*, *Nocardia*, *Amycolatopsis, Mucor* or *Penicillium* genus.

Purity of the active ingredient is important factor for the manufacturing of a safe and effective pharmaceutical. The highest possible purity of the product is especially important if the pharmaceutical product should be taken for a longer period as it is the case in the treatment or the preventing of a high plasma cholesterol. The present invention is related to process for isolation and purification of HMG-CoA reductase inhibitors of purity higher than 99.6%.